Basic structure of the growth cone
Organization of the growth cone.
Fig. 2. (A and B) A model showing one way in which a growth cone might turn toward an attractant (green).
Major Guidance Cues and Their Receptors

Fig. 1. Conserved families of guidance molecules (A) and their receptors (B). Domain names are from SMART (http://smart.embl-heidelberg.de). P1 to P3, DB (DCC-binding), CC1 to CC3, and SP1 and SP2 indicate conserved regions in the cytoplasmic domains of DCC, UNC-5, Robo, and Flexin receptors, respectively.
**Fig. 2: Ephrins and retinotectal patterning**

Regenerating goldfish retinal axons following removal of retinal fields. Anterior (nasal) retina removal, see normal innervation of anterior tectum by posterior (temporal) retinal axons. Complementary result with posterior retina removal. Conclusion: Fixed set of cues that direct topographic connections in visual system. Postulate a gradient of markers with a one-to-one correspondence between axons and their targets. Later studies, however, make this strict interpretation untenable: (1) after several months see expansion of retinal map on tectum following removal of part of the retina; (2) remove a portion of tectum, see after several months a compression of map.

Retinal-tectal specificity: temporal retinal axons are not attracted to anterior tectum, they are repelled from posterior tectum. When temporal retinal axons are allowed to extend over alternating carpets (Bonhoeffer stripe assay) of posterior or anterior tectal membranes, they choose anterior membranes; when posterior membranes are treated with heat or proteases no preference is seen. Ligands for the Eph family of RPTKs (Ephrin A2 and Ephrin A5) are present in a decreasing posterior to anterior gradient in the tectum, while the receptor for Ephrin-A2, EphA3, was found to present in an increasing nasal (anterior) to temporal (posterior) gradient in the retina. Ephrins A2 and A3 can cause growth cone collapse in vitro. Retroviral expression of Ephrin A2 demonstrates a specific inhibitory effect on temporal retinal axons in vivo. Various models are possible for receptor (R) and (L) distributions to generate topographic projections, including single gradient and counter-gradient models employing both repulsive and attractive cues. Nick, the vertebrate orthologue of fly Dock, can interact with Eph receptors, directly linking them to proteins that can alter the cytoskeleton. Additionally, the GEF Ephexin modulate Rho GTPase function and also influence growth cone collapse.
Ephrin signaling and retinotectal mapping

**Anterior-posterior mapping**

(a) Optic tectum/superior colliculus

- Ephrin-A
- EphA (Anterior)
- Retina
- EphA (Nasal)
- Temporal
- Ephrin-A

(b) Growth cone
- Collateral branch
- Future termination zone
- Axon

(c) Posterior
- Termination zone
- Anterior
- Nasal
- Temporal
Ephrin-Eph receptor interactions
FIG. 3

EPHRIN SIGNALING

Ephrin/Eph receptor signaling. EphA receptors interact with GPI-anchored ephrin-As whereas the transmembrane ephrin-Bs bind to EphB receptors. Ephrin/Eph complexes propagate bidirectional signals. Forward signaling: The GEF ephexin links EphA receptors to Rho GTPases. Several additional pathways link ephrin/Eph signaling to regulation of adhesion: Binding of the phosphotyrosine phosphatase Shp-2 to EphA2 leads to inactivation of FAK and decreased integrin-mediated adhesion, whereas increased integrin-mediated adhesion is induced by ephrin-A via a Src-family-dependent pathway and by binding of Nck to activated EphB through the Nck-interacting kinase, NIK. Reverse signaling: Ephrin-As may transduce signals by aggregation with signaling molecules in membrane raft microdomains. SH2-domain-containing adaptor molecules such as Grb4 are recruited to phosphorylated ephrin-Bs. PDZ-RGS3, among other PDZ-binding proteins, binds to ephrin-Bs and may modulate G protein-coupled signaling to ephrins.
Axon guidance in the spinal cord

Figure 2  Different types of cues may collaborate to guide commissural axons to the ventral midline.
(A) Dorsal

- Roof plate
- Commissural neurons
- Floor plate
- Neural cord

Ventral

(B) Netrin
- Slit
- Sema3

Floor plate
Netrin-1 expression in wild type and netrin null mice

Figure 1. Expression of Netrin-1 in Wild-Type and Mutant Mouse Embryos
Axon guidance defects in netrin null mice
DCC is a Netrin receptor

Figure 3. Netrin-1 Binds 293 Cells Expressing DCC but Not TAG-1 or L1
DCC is required for floorplate-induced attraction

Figure 6. DCC Function Is Required for Axon Outgrowth Evoked by Floor Plate Cells
**Figure 6: Netrin Signalling**

Netrin receptor signaling. The netrins are secreted proteins that bind to transmembrane proteins of the DCC family. The composition of netrin receptor complexes dictates the growth cone response to netrin. Binding of netrin to DCC homodimers (left) induces association of DCC cytoplasmic domains, which leads to growth cone attraction. This attraction signal requires elevated levels of cAMP and may involve activation of the Rho GTPases Cdc42 and Rac-1. In contrast, binding of netrin to UNC-5-DCC heterodimers (right) leads to growth cone repulsion.
Slit, Robo, Comm phenotypes

Goodman et al.
Expression of Slit and Robo

Goodman et al
Figure 1. Domain Structures and Homologies of Slit Family Members

(A) Diagram illustrating the domain structures of Slit family members and percent amino acid identities among these members. All identified Slit proteins contain signal peptide (ss), four tandem leucine-rich repeats (LRR), EGF repeats, a conserved ALPS spacer, and a cysteine knot. Both Drosophila Slit and C. elegans Slit lack one LRR in LRR-3 that is present in the three mammalian Slit proteins, and they contain seven EGF repeats compared to nine EGF repeats in the mammalian proteins.

(B) Amino acid sequences corresponding to EGF5 and part of EGF6 of Drosophila Slit (D), rat Slit1 (R1), human Slit2 (H2), rat Slit3 (R3), and C. elegans Slit (C) are shown to illustrate conservation of the putative proteolytic cleavage site. The peptide sequence obtained by microsequencing the amino terminus of the C-terminal cleavage fragment of hSlit2 (Slit2-C) is shown shaded, and the putative cleavage site in hSlit2 is marked by a triangle.
Brose et al. 1999
Figure 6. hSlit2 Can Repel Spinal Motor Axons
Fig. 3. Switching sensitivity at the midline. As they cross the floor plate, vertebrate commissural axons lose sensitivity to the midline attractant, netrin, and acquire sensitivity to Slit and semaphorin repellents. This switch may be mediated in part by silencing of netrin attraction by Slit. Drosophila commissural axons also become sensitive to Slit only after crossing. This appears to reflect Comm’s role in regulating the intracellular trafficking of Robo.
Figure 4  Slit/Robo signaling events. Slit family repulsive guidance cues are ligands for members of the Robo family of transmembrane proteins. Activation of Robo by Slit recruits srGAPs (slit-robo GAPs) to the receptor complex and leads to the inactivation of Cdc42. The profilin-binding protein Ena has also been implicated as an effector of Slit/Robo-induced signals. Downregulation of Robo signaling may be achieved through phosphorylation of its CC1 motif by the Abelson tyrosine kinase (Abl). In addition, surface expression of Drosophila Robo is regulated through its association with Commissureless (Comm). Comm may downregulate Robo expression by targeting Robo protein to the late endocytic pathway.
Semaphorins and their receptors

Semaphorins and their receptors. (a) Semaphorins exist as secreted, glycosylphosphatidylinositol (GPI)-anchored, or membrane-spanning proteins. Subclasses 1 and 2 represent invertebrate semaphorins; subclasses 3–7 are vertebrate semaphorins, and subclass V compose viral semaphorins. (b) Plexins, Otks, neuropilins, L1, Met, CD72 and Tim-2 serve as semaphorin receptors or components thereof: (i) SemaVA, SemaVB, Sema7A and vertebrate class 4 semaphorins directly interact with plexins; (ii) in Drosophila, Sema1a signals repulsion through a PlexA/Otk receptor complex; (iii) class 3 semaphorin receptors are the best characterized group and are composed of neuropilins (the ligand binding unit), plexins (the transducing unit), and L1 (the modulatory unit); (iv) Effects of Sema-A on epithelial cell invasive growth are mediated through a PlexB1/Met receptor complex; (v) CD72 serves as a Sema4D receptor in the immune system; and (vi) Sema4A exerts its effects on T cell function through Tim-2. Domains: α-hel, α-helical coiled-coiled; BD, basic; C1, intracellular 1; C2, intracellular 2; C-type lectin; CUB, complement binding; Cyto, cytoplasmic; Ext, extracellular; Fill, fibronectin type III; FVIII, coagulation factor; G-P, glycosyl-proline-rich; GPI, anchor; Ig, immunoglobulin-like; IgV, immunoglobulin variable region; ITIM, immunoreceptor tyrosine-based inhibitory motif; MAM, ‘Meprin, A5, Mu’; MRS, Met-related sequence; Muc, mucine; PMR, polymorphic region; Sema, semaphorin; SS, signal sequence; TK, tyrosine kinase; TR, thrombospondin.
Effect of Sema3A on sensory neurons
Semaphorins induce growth cone collapse and axon repulsion.
Sema3A binds to Neuropilin-expressing cells

Figure 2. Identification of Neuropilin as a Sema III–AP Binding Protein by Expression Cloning

Tessier-Lavigne et al.
Neuropilin is required for Sema3A-induced axon repulsion

Figure 7. Neuropilin Is Required for Sema III-Evoked Repulsion of NGF-Responsive DRG Axons
E14 rat DRG explants were cultured in collagen gels with 25 ng/ml NGF to elicit outgrowth of Sema III-responsive axons (Messersmith et al., 1995). Explants were cocultured with aggregates of 293-EBNA cells secreting Sema III-AP protein (right in each panel) in the presence of 0 μg/ml (A), 2 μg/ml (B), 4 μg/ml (C), or 10 μg/ml (D) of anti-neuropilin IgG, 10 μg/ml of preimmune IgG (E), or 10 μg/ml of depleted (F) or mock-depleted (G) anti-neuropilin IgG for 40 hr. The explants were then fixed and visualized by wholmount immunostaining with the anti-neurofilament antibody NF-M. DRG neurites proximal to, but not distal to, the Sema III-AP-secreting cells were repelled in the absence of anti-neuropilin antibody, an effect that was blocked in a dose-dependent fashion by addition of the antibody. The amounts of neurite outgrowth from DRG in the proximal and distal quadrants (H) are compared in Table 1. Scale bar: 350 μm.
Semaphorin Signaling

Figure 2
(A) Extracellular matrix molecules

- ECM
- Integrin receptors
- Kinases/other signaling molecules
- Further signal transduction

(B) CAMs

- L1
- NCAM
- Kinases/other signaling molecules